Allelochemicals of Three Amazon Plants Identified by GC-MS

V. Sotero, P. Suarez, J.E. Vela, D. García de Sotero, Y. Fujii

***Abstract*— The aim of this study was to realize the evaluation of the allelopathic activity of 83 vegetable species from Allpahuayo – Mishana Reserve in Peruvian Amazon, and to determine the main polar components of three species of that showed high activity. Leaves samples were collected, which were subjected to elution for two weeks to get the methanol extracts e to test the inhibition of the roots of pre-germinated seeds of *Lactuca sativa*. These extracts were dried in a rotary evaporator and the product subjected to column fractionation opened using silica gel No. 100, using as mobile phase methanol and obtaining the fractions according to the appropriate retention time, and meet the fractions containing similar molecules through analysis of thin layer chromatography; which were tested to evaluate their allelopathic activity against pre-germinated seeds of *Lactuca sativa*. In this way it was found that three species showed activity in extracts, these were the *Iryanthera ulei, Duroia hirsuta and Theobroma obovatum*. When performing the analysis on GC-MS. was found compounds as terpenes, phenolics and organic acids, as the following: isoeugenol, catechol, humulene in *I. ulei*; limonene, geranic acid, neric acid, homovanillil alcohol in *D. hirsute*; phenol. 2,4-bis (1.1-dimethylethyl), α ionone in *T. obovatum* and phytol in each.**

***Index Terms*— Allelopathy, Iryanthera, Theobroma, Duroia, amazonian species**

I. INTRODUCTION

Medicinal plants are becoming integral components of many subsistent farming systems in most developing nations, due to the increasing awareness of human needs for wild indigenous plants as herbal remedies. Contrary to the acclaimed curative effects of several groups of plant's secondary metabolites, accumulation of these organic components in the soil negatively affect seed germination and seedling growth of other vegetations through a phenomenon called allelopathy [1].

Allelopathy was defined as the process in which a plant off the environment, one or more chemical compounds (allelochemicals), that inhibit the growth of other plants that live in the same habitat or in a nearby habitat[2]. Allelopathic interactions are mediated by secondary metabolites released from the donor plants in environment and influence growth and development in natural and agro-ecosystems. Allelochemicals phytotoxic effects are calIed 'alIelochemical stress' [3].

V. Sotero: Research Coordinator of Foundation for the Sustainable Amazonian in Lowland (FUNDESAB), Iquitos-Peru. Telf. 51-65-22-5266,

P. Suarez, J. E. Vela and D. García de Sotero, Research of National University of Peruvian Amazon (UNAP). Iquitos – Peru.

Y. Fujii. Research of University of Agriculture and Technology. Fuchu. Tokyo 183-8509 Japan.

Most of the allelochemicals are actually identified as coumarins, tannins, flavonoids, terpenes and some alkaloids, steroids and quinones [4] and directly affects many physiological and biochemical reactions and thereby, influence the growth and development of plants. The allelochemicals reduces the cell division and have several phytotoxic effects. For example, ferulic acid (It is found in plant cell) stress on plant roots, reduces the water use, inhibits foliar expansion and root elongation, reduces rates of photosynthesis and inhibits nutrient uptake [3]. The phytotoxicity of several Eucalyptus, is due to the presence of various compounds in their essential oils, such as α -pinene, 1, 8- cineol, citronelol, citronella, citronellyl acetate, limonene, linalol, γ-terpinene, *allo*-ocimene and aromadendrene [5]. Similary at *Carex distachya* Desf, were identified flavonids and stilbenoids which possessed potencial alleopatic, among these were found pallidol and tricin between others [6]. *Arctotis arctotoides* showed high allelopatic activity and their chemical composition reported the presence of phenolics, sesquiterpene, monoterpene, coumarins, alkaloids, 1, 8-cineole and limonene in the essential oils of the two plant's parts [1].

In plants, allelochemicals can be present in the leaves, bark, roots, root exudates, flowers, and fruits. These toxins can affect a target species in a number of ways. Many of these have more effects on seed germination than on the growth and viability of adult plants. In fact, catechin from *Cenτrea maculosa* inhibits seed germination in plant species whose seedlings are generally tolerant of its phytotoxic effects seed germination in *Pinus laricio* is inhibited by several phenolic compounds from the soils around *P. laricio* and *Fagus sylvatica* trees. This inhibition has been attributed to a disruption of the activity of metabolic enzymes that are involved in glycolysis and the oxidative pentose phosphate pathway (OPPP), which takes substrates from glycolysis and feeds its products back into glycolysis. Several of these phenolic compounds, such as vanillic, p-coumeric, p-hydroxybenzoic and protocatechuic acid, tested alone and in combinations, were able to inhibit the enzymatic activity of all or several of the enzymes monitored. This suggests that the decrease in enzymatic activity is a secondary effect of these compounds, which might be caused by general protein damage leading to decreased enzymatic activity. Some allelochemicals compounds are thought to interact with the mitochondrial membrane and to impair mitochondrial respiration directly. The monoterpenes camphor, apinene, and limonene all strongly affected the respiratory activity of soybean radicular hypocotyl mitochondria, but apparently have different modes of action [7].

Allelopathy can also be a problem when non-native plant species threatens biodiversity and ecosystem stability This process is responsible for the decline in native species richness and the extinction of certain species of changes in the

relationship between soil microorganisms, changes in the availability of soil nitrogen and other nutrients and changes in soil characteristics [8].

There are many studies focusing on the effects of alleopathy on agroecsystems and on their explotation in agriculture. In sustentable agriculture, the major threat and extremally challenging task is weed control. Weed management is an important part of the following reducing yield crop seeds contamination with weed seeds as well as limiting the buildup of the weed seed bank in the soil. It has recently been suggested in several papers, that allelopathy holds great prospects for finding an alternative strategy for weed mangament whereby by reliance on traditional herbicides in crop production can be reduced as the leaves of sunflower (*Helianthus annuus)* [9], and the hairy vetch (*Vicia villosa* Roth) residue [10].

In this study it was explored three metanolic extracts by Gas chromatography–Mass spectrometry (GC-MS) after the preliminar alleopatic evaluation of 83 plants form the Peruvian amazon: *Iryantera ulei, Duroia hirsuta* and *Theobroma cacao*.

II. MATERIAL AND METHODS

Leaves of 83 plants at good situation, from The Botanical Garden of IIAP in Allpahuayo-Mishana Reserve – Loreto-Peru, were collected the 2010 and 2011 years, they are listed at Table 1, This plants were previously identified by Vasquez & Phillips [11] whose vouchers were deposited in the Herbaria of at National University of Peruvian Amazon (AMAZ) and Missouri Botanical Garden.

Plant materials were washed with distilled water and lixiviated with methanol at the proportion of 5 g in 100 ml of methanol for 15 days, this product is filtered and the product utilized for the previous allelophatic evaluation. With the methanolic extract were realized the bioassays to evaluate their effect on germinated seeds of *Lattuca sativa* [12]. It was impregnated at filter paper (Whatman Nº 1 and 2.7 cm of diameter) and collocated in Petri dishes with 100 µl of the solution to study at concentrations of 10; 3; 1; 0.3 y 0.1 mg/ml. after the evaporation of methanol they were added with 700 µl of distilled water and there was placed five pre germinated seeds of *L. sativa* by 52 hrs (20ºC in dark). Every experiment was realized by triplicate and for the blank; after of this was measurement the length of the roots of every seed and compared with the blank seeds. For the bioassay of the fractions it was similar but only was utilizing 100 µl of everyone.

Extracts dried of the six species with higher allelophatic value, were fractionates in chromatographic column, utilizing as mobile phase methanol and as stationary silica gel Nº 100, the elution was at 20 drops/min and the fractions collected in assay tubes getting's average 100 fractions. This fractions were analyzed at thin layer with silica gel 60 F_{254} (6.7 x 6.2 cm) utilizing as mobile phase methanol, getting of this manner about 10 fractions with molecules with similar Rf, and this were submitted to allelophatic evaluation. Analysis of the fractions with allelophatic activity which were in average of three, were analyzed at Bruker-Scion TQ gas chromatograph coupled with spectrophotometer of mass, utilizing a capillary column of silica BR-5ms of 15 m. ID : 0.25 mµm, with the electrons impact at 70 eV. Gas Helium at constant flux of 1 ml/min and a injection volume of 0.5 and split mode, temperature of 250 ºC, Temperature of oven programmed to 40 ºC increasing at 8 ºC /min until 200ºC., spectrums of mass were got at a 70 eV and the fragments since 35 a 500 m/z. The identification was realized with the NIST library.

III. RESULTS AND DISCUSSION

Accord Table 2, exist 17 species which presented hormesis and the rest have since moderated to high alleopatic activity, and six with higher allelophatic activity, which are presented in the Table 3. They are *Chrysochlanys membranaceae* (Code 11037), *Vitrex triflora* (Code 23075) and *Miconia cazaletti* (Code.24006), Theobroma obovatum (Code 20045) and Iryantera ulei (Code 11001), which EC_{50} are lower than 1.40 mg/ml. After the column chromatographic fractionation eluted with methanol and evaluated the similar R_f substances by thin layer chromatography were collected between 8 to 15 fractions (Table 4). These fractions were subjected to analysis of allelopathy lines by the method indicated above and from these three species that maintained high activity were selected, these were the 11001. 20045 and 23010, as it is showed at Table 5, where besides show the compounds obtained by CG-MS, according to each specie.

a) Specie 11001 (*Iryantera ulei* Warb) belongs to Myristicaceae family, and the species of the Iryanthera genus are used as psychoactive [13]. In this species include the phenolics [(phenol-2-methoxy-4-1(1-propenyl) (isoeugenol)), catechol], and terpenes (ylangene, humulene, cubenol, **α acorenol, t-muurolol, spathuleno, phytol**). Cubenol terpene also was found in the essential oil of erva-de-jabuti (*Peperomia circinnata* Link var. *circinnata*, Piperaceae) [14].

b) Specie 23010 (*Duroia hirsuta* (Poepp.) K.Schum), It has been studied because is known where a group of this specie grows, a zone lacking of vegetation is formed in the Amazon forest and it is known as Devil's gardenc and as result of alleopathy. One study indicates that ant *Myrmelachista schumanni*, which nests in *D. hirsuta* stems, creates Devil's gardens by poisoning all plants except its host plants with formic acid [15],. The principal compounds encountered were iridoid lactone and a flavonol [16], in this study have been encountered acids or its esters (methyl salicilate, linoleico acid ethyl ester, 9, 12, 15-octadecatrienoic acid. methyl ester, n-hexadecanoic acid). Methyl salicilate, terpenes (limonene, geranic acid, neric acid, phytol), organic acids (geranic acid, neric acid), and phenolics (homovanillil alcohol, 1, 3-benzenediol, 4-propyl. In the same way, phenolic compost, as homovanillil alcohol, Specie 20045 (*Theobroma obovatum* Klotsch), presents organic acids or its esters (oleic, hexadecanoic, octadecatrienoic, hexanoic, 12-octadecadienoic acid. methyl ester, butanoic acid, 3-hexenyl ester. [1, 1´-bicyclopropyl]-2-octanoic acid. 2-hexyl-methyl ester), phenolics (phenol 2, 4-bis (1, 1-dimethylethyl, phenol, 4-pentyl) an ionone (α-ionone), and mequinol. The phytol, an acyclic terpene, which has been found in the three species studied and considered a marker of *Leucas aspera* (Lamiaceae), a plant of traditional medicine of India [17].

IV. CONCLUSIONES

Analyses of secondary metabolite compounds, it is necessary to identify the active ingredients present in many plant species, especially using more advanced techniques. Therefore the analysis by GC-MS, it is very useful to identify the methanol extracts of plants

ACKNOWLEDGMENTS

To the Research Institute of Peruvian Amazon (IIAP) and National Institute for Environmental Sciences (NIAES) for the financial and technical support for this study.

REFERENCES

[1] Badmus, A., Afolayan, Alleopathic potential of Arctotis arctotoides (L.f.) O. Hoffm aquops extreacts on the germination andseedling growth of some vegetavles. African Journal of Biotechnology. 11: 10711-10716 (2012).

[2] Molish H. Der Einfluss einer Pflanze auf die andere-Allelopathie, Gustav, Fischer, Verlag and Jena. (1937).

[3] Hussain, S., Siddiqui, SU, Khalid, S, Jamal A, Qayyum, A, Ahmad, Z. Allelopathic potential of Senna (*Cassia angustifolia* Vahl.) on germination and seedling characters of some major cereal crops and their associated weeds. Pak. J. Bot., 39: 1145-1153 (2007).

[4] Phippen, W.B., Simon, J.E. Anthocyanin inheritance and instability in purple basil (*Ocimum basilicum* L.). Journal of Heredity. 91:289–296 (2000).

[5] Gonzales Castillo, L. Efecto del aceite esencial y extractos acuosos de *Eucalyphus gomphocephala*, sobre la germinación y crecimiento de arvensis. Tesina. Universitat Politecnica de Valencia. pp. 20-26 (2011).

[6] Fiorentiono, A., D'Abrosca,B.,Pacifico, S., Izzo, A., Letizia, M.,Esposito, A.; Monacoi, P. Potential allelopatic effects of stilbenoids and flavonoids from leaves of Carex distachya Desf. Biochemical Systematics and Ecology. 36: 691–698 (2008)

[7] Weir, L., Park. S.,Vivanco, J. Biochemical and physiological mechanisms mediated by allelochemicals Current Opinion in Plant Biology.7:472–479 (2004).

[8] Lorenzo, P.; Gonzales, L. Alelopatía: una característica ecofisiológica que favorece la capacidad invasora de las especies vegetales Ecosistemas 19: 79-91(2010).

[9] Gawronska, H., Ciarka D., Bernat, W., Gawronski S.W. Sunflower – desired allelopathic crop for sustainable and organic agriculture? In: Fujii Y., Hiradate S. (eds) New Concepts and Methodology in Allelopathy. Science Publishers, Inc. Enfield, NH, USA.:pp. 185-210.(2007).

[10] Teasdale, J., Abdul-Baki, A., Park, Y., Rosecrance, R. The potential for alleopathy during decomposition of heavy vetch residue In: Fujii Y., Hiradate. S. (eds) New Concepts and Methodology in Allelopathy. Science Publishers, Inc. Enfield, NH, USA. pp. 211-225 (2007).

[11] Vasquez, R.; Phillips, O. Allpahuayo: floristics, structure, and dynamics of a high-diversity forest in Amazonian Peru. Annals of the Missouri Botanical Garden, 87: 499-527 (2000).

[12] Fujii, Y., Shibuya,T., Yasuda, T. Methods for screening allelopathic activities by using the logistic function (Richards' function) fitted to lettuce seed germination and growth curves. Weed Research, 35: 353-361 (1990).

[13] Neill, D., Baker, H., Palacios, H., Zaruma, J. Plant resources of Amazonian Ecuador. Flora del Ecuador. Missouri: Missouri Botanical Garden. Second annual report. AID. Dirección Nacional Forestal, pp. 449-527 (1987).

[14] Maia, J., Andrade, E. Database of the Amazon aromatic plants and their essential oils. Química Nova. 32: 595-622 (2009).

[15] Frederickson, M., Greene, M., Gordon, D. Devil's gardens' bedevilled by ants. Nature. 437:498 (2005).

[16] Aquino, R.D., Tommasi, N., Tapia,M., Lauro,M., Rastrelli, L. New 3-Methyoxyflavones, an Iridoid Lactone and a Flavonol from *Duroia hirsute*. Journal of Natural Products, 62: 560-562 (1999).

[17] Basarkar, U.G. Phytol –A chemical constituent of *Leucas aspera* through HPTLC. American International Journal of Research in Formal, Applied & Natural Sciences. 14: 44-46 (2014)

Table 1. List of species selected and collected at 2010 and 2011 years

2010 year			2011 year		
CODE	**NAME**	**FAMILY**	**CODE**	**NAME**	**FAMILY**
6025	*Socratea exorrhiza* (Mart.) H. wendell	Arecaceae	6029	*Miconia amazónica* Triana	Melastomataceae
6027	*Astrocaryum macrocalyx* Burret	Arecaceae	6042	*Cecropia sciadophylla* Mart	Urticaceae
6073	*Eschweilera coriácea* (DC.)S.A. Mori	Lecythidaceae	9022	*Iryanthera crassifolia* A.C. Sm.	Myristicaceae
6077	*Isertia hypoleuca* Benth	Rubiaceae	9079	*Phytelephas macrocarpa* Ruiz & Pav.	Arecaceae
9006	*Pentagonia macrophylla* Benth	Rubiaceae	9091	*Caryodendron orinocense H. Karst.*	Euphorbiaceae
9025	*Diospyros tessmannii* Hiern	Ebenaceae	10073	*Guarea guentheri Harms.*	Meliaceae
9031	*Calyptranthes plicata* Mc Vaugh	Myrtaceae	23014	*Warszewiczia coccínea (Vahl) Klotzsch.*	Rubiaceae
9032	*Tetrathylacium macrophyllum* Poepp & Endl	Salicaceae	23075	*Vitex triflora Vahl*	Lamiaceae
9051	*Rinorea lindeniana* (Aubl.) Kuntae	Violaceae	24022	*Tetrastylidium peruvianum Sleumer.*	Olacaceae
9076	*Otoba parvifolia*	Myristicaceae	24048	*Pseudoxandra polyphleba (Diels) R. E. Fr.*	Annonaceae
10033	*Iriartea deltoidea* Ruiz & Pav.	Arecaceae	24108	*Diospyros subrotata Hiern.*	Ebenaceae
10034	*Rinorea guianensis*	Violaceae	41024	*Maquira calophylla (Poepp. & ENDL.)*	Moraceae
10060	*Gloeospermum sphaerocarpum*	Violaceae	41032	*Neea macrophylla* Poepp. & Endl.	Nyctaginaceae
10090	*Pouroma cecropiifolia*	Urticaceae	41047	*Croton cuneatus Klotzsch.*	Euphorbiaceae
10117	*Cassipourea peruviana* Alston	Rhizophoraceae	59058	*Cecropia engleriana Snethl.*	Urticaceae
11001	*Iryanthera ulei* Warb.	Myristicaceae	63096	*Matisia bracteolosa Ducke.*	Malvaceae
11012	*Neea verticillata* Ruiz y Pav.	Nyctaginaceae	69009	*Theobroma speciosum Willd.*	Sterculiaceae
11035	*Allophylus loretensis*	Sapindaceae	69034	*Zygia basijuga (Ducke) Barneby & J. W. grimes.*	Fabaceae
11037	*Chrysochlamys membranácea* Pland & Triana	Clusiaceae	69036	*Annona excellens R. E. Fr.*	Annonaceae
11078	*Cestrum megalophyllum* Dunal	Solanaceae	69043	*Protium ferrugineum* (Engl.) Engl.	Burseraceae
11082	*Euterpe precatoria* Mart.	Arecaceae	69072	*Leonia cymosa Mart.*	Violaceae
11097	*Pourouma guianensis* Poeep. & Endl.	Urticaceae	76055	*Nectandra cissiflora Nees.*	Lauraceae
20005	*Neea divaricata* Poepp. & Endl.	Nyctaginaceae	77003	*Micropholis egensis* (A.D.C.) Pierre	Sapotaceae
20029	*Crepidospermum goudotianum* (Tul.)	Burseraceae	77015	*Matisia ochrocalyx K. schum*	Malvaceae
20045	*Theobroma obovatum* Klotsch ex Bernoulli	Malvaceae	77073	*Banara nítida Spruce Ex Berth*	Salicaceae
20104	*Lacistema aggregatum* (Bergius) Rusby	Lacistemataceae	78014	*Hirtella racemosa var racemosa Baardseth*	Chrysobalanaceae
20107	*Guatteria puncticulata* R.E.Fr	Annonaceae	78049	*Leonia glycycarpa* Ruiz & Pav. (var glycicarpa)	Violaceae
20122	*Pouteria cuspidata* (A.D.C.) Baehni	Sapotaceae	78063	*Leonia crassa*	Violaceae
20125	*Huertea glandulosa* Ruiz & Pav.	Staphyleaceae	79002	*Unonopsis spectabilis* Diels	Annonaceae
23010	*Duroia hirsuta* (Poepp.) k. Schum	Rubiaceae	79006	*Matisia malacocalyx*	Malvaceae
24006	*Miconia cazaletti* Wurdack	Melastomataceae	79011	*Macrolobium limbatum Spruce ex Benth. Var limbastum*	Fabaceae
24047	*Guarea gomma* Pulle	Meliaceae	79013	*Inga cf. Brachyrhachis Harms.*	Fabaceae
41081	*Iryanthera laevis* Markgr.	Myristicaceae	79018	*Trigynaea duckei (R. E. Fr.) R. E. Fr.*	Annonaceae
41082	*Siparuna cristata* (Peopp. & Endl.)	Monimiaceae	79089	*Tabernaemontana sanano*	Apocynaceae
42070	*Unonopsis peruviana* R.E.Fr	Annonaceae	80028	*Cecropia membranácea Trécul.*	Urticaceae
59042	*Pourouma minor* Benoist	Urticaceae			
59044	*Naucleopsis naga* Pitter	Moraceae			
59045	*Pseudolmedia laevis* (Ruiz y Pav.) J	Moraceae			
59087	*Guapira noxia* (Netto) Lundl	Nyctaginaceae			
59141	*Lindackeria paludosa breviflora* Croat	Achariaceae			
60015	*Cathedra acuminata* (Benth.) Miers	Olacaceae			
60049	*Turpinia occidentalis* breviflora Croat	Staphyleaceae			
60064	*Siparuna decipiens* A.dc	Monimiaceae			
60074	*Rinorea flavescens* (Aubl.) Kuntae	Violaceae			
60112	*Apeiba aspera* (Aubl.)	Tiliaceae			
60141	*Guarea pterorhachis* Harms	Meliaceae			
60149	*Pouteria torta tuberculata* (Steumer)	Sapotaceae			
60159	*Siparuna bífida* A.dc.	Monimiaceae			

Table 2. Inhibitory percentage of the root of *Lattuca sativa* at different concentrations of the extracts of 83 species evaluated

	Species Code															
mg/mL	**6025**	**6027**	**6073**	**6077**	**9006**	**9025**	**9031**	**9032**	**9051**	**9076**	**10033**	**10034**	**10060**	**10090**	**10117**	**11001**
0,1	-11	-11	20	16	-11	-9	-13	-12	-15	11	0	3	7	7	3	40
0,3	-17	-3	11	20	-2	3	-15	4	-18	13	0	15	27	17	8	35
1	-13	-10	13	40	2	21	-22	-10	-5	7	-2	21	32	6	19	44
3	-18	-3	40	50	3	47	-15	-12	-1	36	9	41	63	38	50	75
10	14	5	80	81	9	63	-3	-12	15	86	4	70	83	55	68	81
mg/ml	**11012**	**11035**	**11037**	**11078**	**11082**	**11097**	**20005**	**20029**	**20045**	**20104**	**20107**	**20122**	**20125**	**23010**	**24006**	**24047**
0,1	15	-11	31	13	-1	3	-6	23	7	9	-31	24	4	11	58	19
0,3	21	2	51	-2	8	14	1	3	33	16	3	21	1	35	32	20
1	6	13	90	17	-4	13	11	23	51	10	-26	26	19	45	88	43
3	33	19	93	16	12	32	44	36	64	23	9	44	43	63	90	63
10	43	13	94	7	25	53	64	35	80	37	78	80	51	82	93	62
mg/ml	**41081**	**41082**	**42070**	**59042**	**59044**	**59045**	**59087**	**59141**	**60015**	**60049**	**60064**	**60074**	**60112**	**60141**	**60149**	**60159**
0,1	10	-1	3	-9	-11	-19	-11	-9	-10	-14	-26	-17	-16	-	4	-16
0,3	27	8	-8	-5	7	15	1	-14	6	0	-1	-23	-9	-	-15	-5
1	22	-4	14	6	-5	26	-8	-11	23	-11	-11	-19	-1	32	-3	10
3	51	12	9	-3	4	43	7	13	46	-1	-1	-7	-23	47	-9	8
10	81	15	10	19	10	43	10	17	54	3	21	4	3	-	9	17
mg/mL	**6029**	**6042**	**9022**	**9079**	**9091**	**10073**	**23014**	**23075**	**24022**	**24048**	**24108**	**41024**	**41032**	**41047**	**59058**	**63096**
0,1	16	7	-30	-6	-6	-20	1	37	19	28	8	9	-4	-3	12	9
0,3	13	10	-25	-7	-22	-35	22	48	14	-1	7	-12	1	-6	15	15
1	14	12	-7	-2	-21	-10	26	64	12	1	11	2	-6	0	6	12
3	34	-6	-20	17	-22	-16	25	65	16	9	9	6	-8	11	14	18
10	60	15	10	36	2	44	42	65	37	55	20	-11	6	9	27	19
mg/mL	**69009**	**69034**	**69036**	**69043**	**69072**	**76055**	**77003**	**77015**	**77073**	**78014**	**78049**	**78063**	**79002**	**79006**	**79011**	**79013**
0,1	16	7	6	21	7	4	-2	16	22	6	12	6	10	0	12	3
0,3	15	7	13	11	15	7	3	9	20	13	11	13	8	8	-4	9
1	20	6	10	16	20	23	27	6	16	30	2	-2	-42	6	-8	-8
3	10	17	28	15	17	22	12	26	12	20	20	11	13	11	15	-1
10	21	20	17	44	19	22	30	68	23	32	3	6	-19	53	19	12
mg/mL	**79018**	**79089**	**80028**													
0,1	-22	-2	13													
0,3	-4	-19	9													
1	-16	-19	20													
3	-12	-5	10													
10	33	-8	17													

Table 3. Six species evaluated with major alellophatic activity

Nº	Code	Family	Genus	Specific epithet	EC$_{50}$
1	11037	Clusiaceae	Chrysochlanys	membranaceae	0.29
2	23075	Lamiaceae	Vitex	triflora	0.35
3	24006	Melastomataceae	Miconia	cazaletti	0.45
4	20045	Sterculiaceae	Theobroma	obovatum	0.90
5	11001	Myristicaceae	Iryanthera	ulei	1.30
6	23010	Rubiaceae	Duroia	hirsuta	1.40

Table 4. Number of methanolic fractions and retention time in every specie and recolected according to the results of thin layer chromatography

Fraction	Spècies					
	11001	**11037**	**20045**	**23010**	**23075**	**24006**
F1	0- 1:50 h	0-1:20 h	0-1:40 h	0- 1:30 h	1-10:40h	0-1:50 h
F2	1:50-2:20 h		1:40-2:20h	1:30-2:10 h	1:40-2:00 h	1:50-2:20 h
F3	2:20-3:30h	2:20-3:20h	2:20-2:40 h	2:10- 2:30 h	2:00-2:10 h	2:20-3: 30 h
F4	3:30-4:40 h	3:20-3:40h	2:40-3:40h	2:30-3:10h	2:10- 2:40h	3:40-4:40 h
F5	4:40-5:20 h	3:40-4:20 h	3:10-3:30h	3:10-4:10 h	2:40-3:00 h	4:40-5:20h
F6	5:20-6:20 h	4:20-4:50h	3:10 h-4:10 h	4:10 h- 4:50 h	3:00h- 4:10 h	5:20 h-6:20 h
F7	6:20-6:50 h	4:50- 5:20 h	4:10-5:10 h	4:50-5:20 h	4:10 h-4:50 h	6:20 h-6:50 h
F8	6:50-9:50 h	5:20 h-6:30 h	5:10- 7:10 h	5:20-6:20 h	4:50-5:40 h	6:50 h-9:50h
F9		6:30-7:20 h	7:10- 9:10 h	6:20 h- 7:30 h	5:40- 6:50 h	
F10		7:20-8:20 h		7:30- 7:50 h	6:50 h-7:50 h	
F11		8:20-9:50 h		7:50-8:30:h	7:50 h-8:50 h	
F12		9:50-10:20h		8:30 h-9:40 h	8:50-10:20 h	
F13		10:20-11:40 h			10:20 h-11:00 h	
F14		11:40-12:40 h			11:00- 11:30 h	
F15					11:20-12:10 h	

Table 5. Molecules probable encountered at the fractions with allelopathic activity analyzed with CG –MS.

Retention time. min	**Compounds**	**Retention time. min**	**Compounds**	**Retention time. min**	**Compounds**
Specie 11001 (*Iryantera ulei* Warb)					
	F2		**F3**		**F4**
14.905	Undecane. 3.6-dimethyl	5.335	Ethanol. 2-butoxy	5.321	Ethanol. 2-butoxy-
16.607	1H-Indene. 1-methylene-	6.445	Propanedioic acid. dimethyl ester	21.137	Caryophyllene
17.3	Decane.2.4.6-trimethyl	17.22	Catechol	21.332	Γ.-elemene
21.118	β- ylangene	19.285 20.317	2-methoxy-4-vinylphenol A.-ylangene	21.605 21.722	Phenol. 2-methoxy-4-(1-propenyl)- Humulene
21.508	α-ylangene	20.921	Phenol. 2-methoxy-4-(1-propenyl)	22.131	β- copaene
21.605	Phenol. 2-methoxy-4-(1-propenyl)	21.135	α.-ylangene	22.364	Γ.-elemene
21.703	Humulene	21.447	α-guaiene	22.89	Benzenepropanoic acid. 2-methoxy-.methyl ester $C_{11}H_{14}O_3$
22.501	β-copaene	22.148	β.-copaene	23.572	(-)-Spathulenol
22.677	epi-cubenol	22.362	Γ.-elemene	23.747	Alloaromadendrene
23.3	Γ-elemene	22.733	Naphtalene. 1. 2. 3. 5. 6. 8a-hexahydro-4.7-dimethyl-1-(1-methylethyl)-. (1S-cis)	23.903	Guaiol
23.572	(-)-Spathulenol	23.57	(-)-Spathulenol	24.156	Cubenol
23.748	α.-acorenol	23.921	Ledol	24.585	T.-cadinol
24.313	12.15-Octadecadienoic acid. methyl ester	24.154	Cubenol	24.721	A.-cadinol
24.469	Bicyclo[4.4.9]dec-1_ene. 2-isopropyl-5-methyl-9-methylene	24.719	A.-cadinol	27.896	Alloaromadendrene oxide-(2)
24.703	T-muurolol	24.914	T.-cadinol	30.545	9.12.15-octadecatrienoic acid. methyl ester. (Z.Z.Z)
25.775	Isoaromadendrene epoxide	25.615	Isoaromadendrene epoxide	30.779	Phytol
27.275	1-Heptatriacotanol	28.4	Benzenepropanoic acid.3.5-bis(1.1-dimethylethyl)-4-h	31.169	2-Methyl-Z.Z-3.13-octadecadienol
27.301	Pregan-20-one. 2-hydroxy-5.6-epoxy--15 methy	30.816	Phytol	33.409	3-Amino-4-[4-hydroxyphenyl]butanool
28.391	Benzenepropanoic acid.3.5-bis(1.1-dimethylethyl)-4-hydroxy-.methyl ester			34.266	Benzene. 1.1´-(1.2-ehtanediyl)bis[2.3.4.5.6-pentamethyl

28.411	Hexadecanoic acid. methyl ester				
28.917	n-Hexadecanoic acid				
30.534	9.12.15-Octadecatrienoic acid. methyl ester. (Z.Z.Z)				
30.748	Phytol				
	Specie 23010 (*Duroia hirsuta* (Poepp.) K. Schum)				
	F2		**F3**		**F4**
16.925	Methyl saliciylate	16.939	Methyl salicilate	16.925	Methyl salicylate
30.715	Phytol	20.192	Geranic acid	20.177	Neric acid
31.3	R-liimonene	23.211	Homovanillil alcohol	23.177	Homovanillil alcoholo
		24.477	1.3-Benzenediol. 4-propyl	30.481	9.12-Octadecadienoic acid. methyl ester. (E.E)
		30.732	Phytol	31.069	Linoleic acid ethyl ester
		31.083	9.12.15-ctadecatrienoic acid. methyl ester. (Z.Z.Z)		
	F5	**F6**			
16.928	Methyl salicylate	28.415	Hexadecanoic acid. methyl ester		
28.868	n-Hexadecanoic acid	28.882	n-Hexadecanoic acid		
30.718	Phytol	30.499	9.12.15-Octadecatrienoic acid. methyl ester. (Z.Z.Z)		
31.069	Linoleic acid ethyl ester	30.85	2-Hydroxyhexadecyl butanoate		
	Specie 20045 (*Theobroma obovatum* Klotsch)				
	F4		**F5**		**F6**
23.917	9-octadecene	11.564	Hexanoic acid	16.603	Naphthalene
26.293	Oleic acid	14.261	Mequinol	21.239	A-ionone
26.741	5-eicosene	16.628	Azulene	22.622	Phenol. 2.4-bis(1.1-dimethylethyl)
27.306	R-limonene	16.979	butanoic acid. 3-hexenyl ester. E	25.758	Methyl tetradecanoate
27.871	2-Methyl-Z.Z-3.13-octadecad ienol	17.057	1- α - terpineol	27.26	9-Tetradecene-1-ol. acetate. (Z)
29	n-Hexadecanoic acid	17.641	Benzaldehyde 2-methyl		
30.481	9.12-Octadecadienoic acid. methyl ester	21.225	A-ionone		
30.831	Phytol	25.627	Octadecanal		
31.182	9.12.15-Octadecatrienoic acid. (Z.Z.Z)-	23.328	Oleic acid		
33.403	Phenol. 4-pentyl	27.127	Cyclopentanetridecanoic acid. methyl ester		
34.065	Benzene. 1.1-(1.2-ethanediyl)bis [2.3.4.5.6-pentamethyl	27.244	[1.1´-Bicyclopropyl]-2-octanoic acid. 2´-hexyl-. methyl ester		
		27.828	3.7.11.15-Tetramethyl-2-hexadece n-1-ol		
		28.062	7-Hexadecenoic acid. methyl ester. (Z)		
		29.795	8.11.14-Eicosatrienoic acid. (Z.Z.Z)-		
		30.847	Phytol		